Azolla fertilizer as an alternative N source for red spinach production on alluvial and peat soils in West Kalimantan, Indonesia

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Abstract
Food security is an important goal in Indonesia, and each household is expected to utilize their backyard to provide food (vegetables) for their family. One approach to intensify vegetable production is through locally-grown fertilizer. Through biological nitrogen fixation, the *Azolla-Anabaena* symbiosis can provide N for vegetable production. The objective of this study was to evaluate the contributions and Nitrogen Use Efficiency (NUE) of *A. pinnata* as a biofertilizer on red spinach production on Inceptisols and Histosols in West Kalimantan, Indonesia compared to commonly-used fertilizers. The experimental design was a Randomized Complete Block Design with three replications. Five N fertilizer treatments were used: control, urea at 50 kg ha^{-1}, chicken manure at 5 t ha^{-1}, *Azolla* at the urea N rate, and *Azolla* at the manure N rate. Treatment means were compared using the honestly significant difference Tukey adjusted post hoc test (n=3, P<0.1). Overall, the results showed that Manure had the highest yield in the alluvial soil and it was comparable to Urea; whereas, in the peat soil, the significantly highest yield was also from the Manure treatment, but it was comparable to *Azolla* applied at the manure N rate. The highest leaf N content was in the Manure treatment (in the alluvial soil) and the *Azolla* applied at the manure N rate (in the peat soil). The highest NUE was in the Urea treatment in the alluvial soil. Therefore, *Azolla* applied at the manure N rate can be used as an alternative biofertilizer, especially for peat soil.

Key Words
locally-grown, nitrogen, *Azolla*, spinach, alluvial, peat

Introduction

Food security is a substantial goal of the Ministry of Agriculture in Indonesia. One of the food sources encouraged to be grown to improve the national food security are vegetable crops. It is expected that every household in Indonesia can utilize their backyard to provide food (vegetables) for their family (IAARD, 2014). Vegetables provide nutrients that are vital for health and maintenance of the human body. People who eat more vegetables and fruits as part of an overall healthy diet are likely to have reduced risk of some chronic diseases.

One approach to intensify vegetable crop production and improve soil fertility is by using locally-grown fertilizer in vegetable crop fields. This sustainable fertilization could be accomplished through the process of biological nitrogen fixation. Much interest has been generated in enhancing the nutrient status of rice soils by enrichment with diazotophic cyanobacteria and symbiotic systems such as *Azolla*, a water fern which harbours cyanobacteria in its leaf cavities (Prasanna et al., 2012; Vaishampayan et al., 2001). The ability of the endosymbiont *Anabaena azollae* to fix atmospheric nitrogen inside leaf cavities of *Azolla* has made the association useful in rice ecosystems.

Free-living cyanobacteria contribute an average of 15-30 kg N ha^{-1} yr^{-1} (Watanabe and Cholitkul, 1979; Stevenson, 1982; Vaishampayan et al., 2001; Prasanna et al., 2012); whereas, the value goes up to 312 (Stevenson, 1982; Kannaiyan, 1985a) to 600 kg N ha^{-1} yr^{-1} for *Azolla-Anabaena* in rice ecosystems (Vaishampayan et al., 2001; Prasanna et al., 2012). *Azolla* also supplies 150-300 tons ha^{-1} yr^{-1} of green manure that sustains soil microbial growth (Kannaiyan, 1985b).

Studies on *Azolla* utilization have mostly been conducted on rice or other grain crops, such as corn (Ferrera-Cerrato and Romero, 1982), rice-wheat system (Kolhe and Mittra, 1990), and wheat (Nain et al., 2010), in addition to taro (Wagner, 1997; Teckle-Haimanot, 1995), vegetables, and banana (Van Hove, 1989). However, still little is known about utilizing *Azolla* as a biofertilizer in vegetable cropping systems. Therefore, this study was undertaken to evaluate the contributions of *A. pinnata* as a biofertilizer on red spinach production on Inceptisols and Histosols in West Kalimantan, Indonesia compared to commonly-used fertilizers in enhancing vegetable crop yields and other agronomic parameters.

Methods

Study site description

Field studies were conducted in 2015 in alluvial and peat soils in West Kalimantan, Indonesia. The alluvial soil (Inceptisols) in tidal lands agro-ecosystem was located on the Agricultural Research Station of the Assessment Institute for Agricultural Technology in Pal IX Village, Sei Kakap, Kubu Raya Regency. The peat soil (Histosols) was located in Sei Selamat Village, Siantan, Pontianak. The climate is tropical moist (IIIC and IVC) with average temperatures above 18 ^{o}C year-round and average relative humidity is 80.8%. The annual precipitation ranges from 2000-4000 mm (Rejekiningrum et al., 2012).

Materials

Azolla pinnata was used for this study, since this *Azolla* species is native to Indonesia. The *Azolla* nursery was prepared in an artificial pond lined with polyethylene and in some natural ponds located close to the field sites. Plant ash was applied at 0.75 t ha^{-1} into the peat water to increase pH. The water resource used for the *Azolla* nursery and for watering the plants was from natural surface water or rain water. The inoculation rate of *Azolla* for the production ponds was 100-200 g m^{-2}. *Azolla* was harvested three to four weeks after inoculation.

Cultivation

Manual tillage was used for soil cultivation. Plant ash was applied 3 t ha^{-1} after tillage in the peat soil to increase soil pH. The spinach variety used was Red "Giti" Spinach from the Indonesian Vegetable Research Institute. Spinach seedlings were transplanted 21 days after germination using a 20x15 cm planting distance. The crop was watered every two days or less depending on rainfall. Urea was applied in a split application (3 and 14 days after transplanting). Manure (3.19% N) and *Azolla* (2.88% N) were applied 3 days after transplanting. Spinach was harvested 24 days after transplanting.

Experimental design, data collection, and analysis

A Randomized Complete Block Design with three replications was used. There were five treatments: control, urea at 50 kg ha^{-1}, chicken manure at 5 t ha^{-1}, *Azolla* at the urea N rate of 23 kg N ha^{-1}, and *Azolla* at the manure N rate of 108 kg N ha^{-1}. The parameters measured in this study were yield, plant height, leaf and branch numbers, leaf N content, and Nitrogen Use Efficiency (NUE). NUE was defined as the difference in yield between a treatment and control divided by the N application rate. Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was performed on the data by using the Mixed procedure (Proc Mixed). Treatment means were compared using the honestly significant difference (HSD) Tukey adjusted post hoc test (n=3, $P<0.1$).

Results

In general, yield, plant height, leaf and branch numbers, N concentration, and plant N uptake were higher in the alluvial soil than in the peat soil. The highest yield was shown in the Manure treatment in both alluvial and peat soils, i.e. 27.1 and 18.3 t ha^{-1}, respectively, and it was significantly different from the Control (no N fertilizer). However, it was not significantly different from Urea (in the alluvial soil) or *Azolla* at the manure N rate (in the peat soil) (Figure 1).

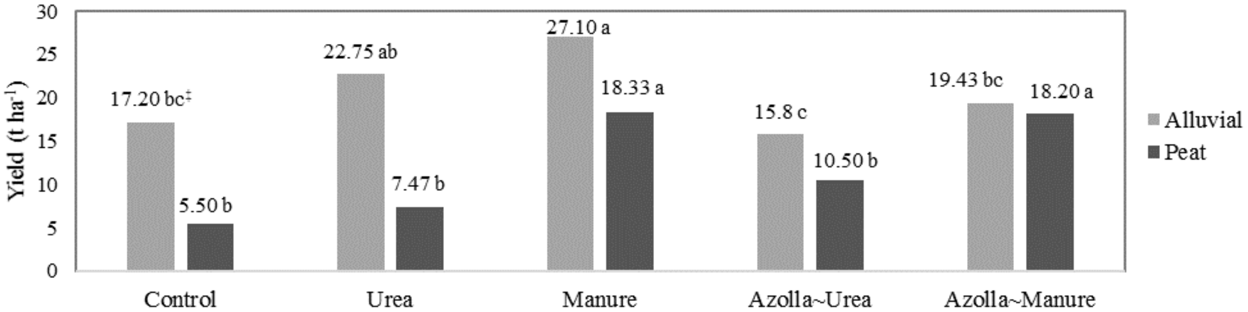

Figure 1. Spinach yield in alluvial and peat soils with different fertilizer treatments.
‡ Values followed by a different letter indicate significant difference within the same soil based on the least square means with a Tukey test ($p<0.1$). '*Azolla*~Urea' = *Azolla* at the urea N rate, '*Azolla*~Manure' = *Azolla* at the manure N rate.

The highest plant height was measured under *Azolla* at the manure N rate in the peat soil, but it was not significantly different from the Manure treatment (Figure 2).

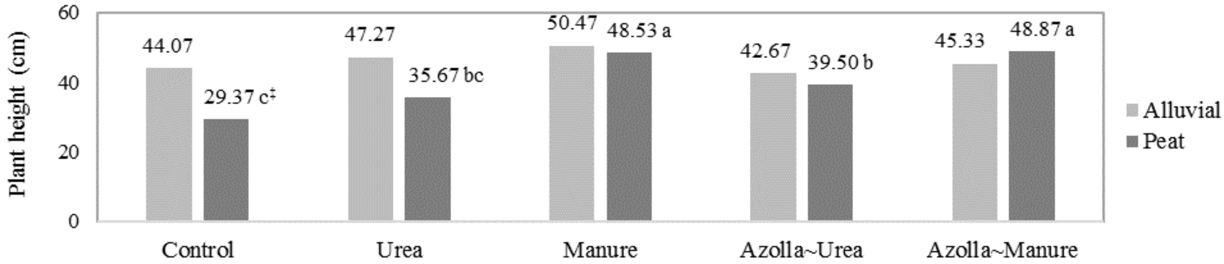

Figure 2. Plant height of spinach grown in alluvial and peat soils with different fertilizer treatments.
‡ Values followed by a different letter indicate significant difference within the same soil based on the least square means with a Tukey test (p<0.1). '*Azolla*~Urea' = *Azolla* at the urea N rate, '*Azolla*~Manure' = *Azolla* at the manure N rate.

Height, leaf and branch number were not significantly different among fertilizer types and control in the alluvial soil, although in general they showed higher values than in the peat soil, i.e. 54.5-69.1, 9.3-11.3, for leaf and branch number, respectively. Interestingly, *Azolla* at the manure N rate demonstrated the significantly greatest leaf and branch number in the peat soil. This was comparable (not significantly different) to other fertilizer types (*Azolla*~Urea, Urea, and Manure) in leaf number and *Azolla*~Urea and Manure fertilizers in branch number; nevertheless, it was significantly different from the Control (leaf number) and from the Control and Urea (branch number) treatments (Figure 3).

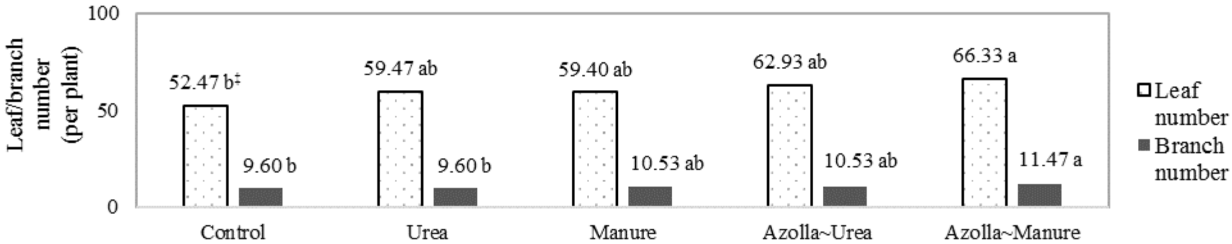

Figure 3. Leaf and branch number of spinach plants grown in peat soil with different fertilizer treatments.
‡ Values followed by a different letter indicate significant difference within the same soil based on the least square means with a Tukey test (p<0.1). '*Azolla*~Urea' = *Azolla* at the urea N rate, '*Azolla*~Manure' = *Azolla* at the manure N rate.

Leaf N content in spinach was higher in alluvial soil (106.0-179.0 kg N/ha) than in the peat soil (65.6-130.3 kg N/ha). The Manure treatment had the highest leaf N content in alluvial soil. *Azolla* at manure N rate showed the highest leaf N content in the peat soil, in contrast, it displayed the lowest N content in the alluvial soil (Figure 4).

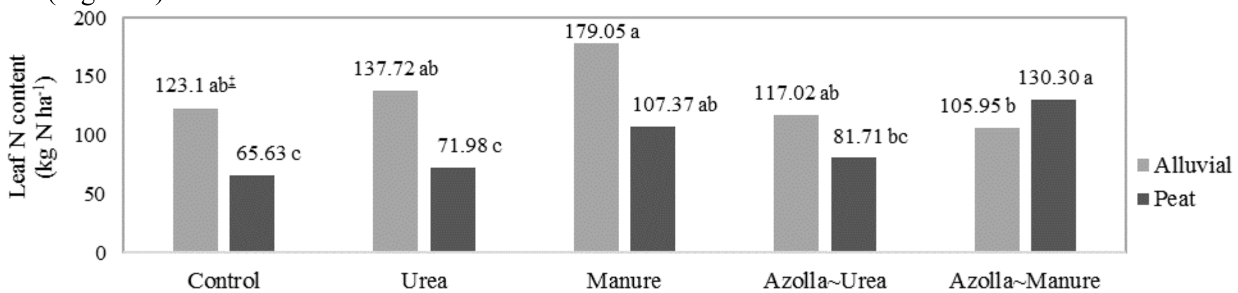

Figure 4. Spinach leaf nitrogen content among fertilizer types on alluvial and peat soils.
‡ Values followed by a different letter indicate significant difference within the same soil based on the least square means with a Tukey test (p<0.1). '*Azolla*~Urea' = *Azolla* at the urea N rate, '*Azolla*~Manure' = *Azolla* at the manure N rate.

There were no significant differences in NUE among fertilizer types in the peat soil, whereas in the alluvial soil, the Urea treatment had a significantly highest NUE, and the Manure, *Azolla*~Urea, and *Azolla*~Manure treatments were not significantly different from each other (Figure 5).

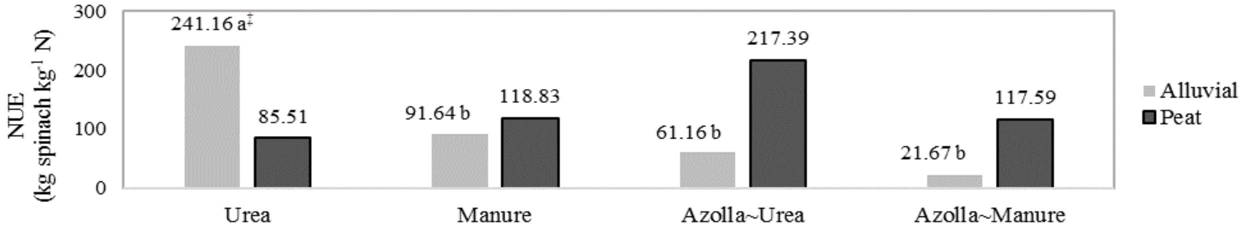

Figure 5. Nitrogen use efficiency in several fertilizer types on alluvial and peat soils.
‡ Values followed by a different letter indicate significant difference within the same soil based on the least square means with a Tukey test ($p<0.1$). '*Azolla*~Urea' = *Azolla* at the urea N rate, '*Azolla*~Manure' = *Azolla* at the manure N rate.

Conclusions

In conclusion, this study showed that:

1. There were higher spinach yields and other agronomic parameters in the alluvial soil than in the peat soil.
2. Manure had the highest yield in the alluvial soil, and it was statistically equal to Urea; whereas, in the peat soil, the highest yield was also in the Manure treatment and it was statistically equal to *Azolla* applied at the manure N rate.
3. The greatest leaf N content was shown in the Manure treatment (in the alluvial soil) and in *Azolla* applied at the manure N rate (in the peat soil).
4. The highest NUE was indicated in the Urea treatment in the alluvial soil, and it was significantly different from the Manure and *Azolla* treatments. There was no significant difference in NUE in the peat soil.
5. *Azolla* applied at the manure N rate can be used as an alternative biofertilizer, especially for peat soil.

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